BIOETHANOL PRODUCTION FROM SUGAR CANE MOLASSES

WAN MUHD FARID BIN WAN HAMAT

A thesis submitted in fulfillment of the
requirements for the award of the degree of
Bachelor of Chemical Engineering (Gas Technology)

Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang

18th JUNE 2012

ABSTRACT

The use of biofuels as an alternative to fossil fuels has expanded in the last few decades. In recent years, growing attention has been devoted to the conversion of biomass into ethanol fuel, which is considered as the cleanest liquid fuel alternative to fossil fuels. Production of ethanol (bioethanol) from biomass is one way to reduce both consumption of crude oil and environmental pollution. An analysis of the current situation and perspective on biomass-to-ethanol is provided in this study. Various conversion pathways are compared from technical, economic, and environmental points of view. This study also deals mainly with the yield of ethanol from molasses with respect to the dilution ratio and the amount of yeast used for fermentation keeping the temperature and fermentation duration constant. Sugarcane molasses is be used for the feedstock in this study. Sugarcane molasses is a viscous by-product of the processing of sugar cane into sugar. Therefore, sugarcane molasses such as agricultural wastes are attractive feedstock for bioethanol production. Agricultural wastes are cost effective, renewable and abundant. In this study, the yeast used is Saccharomyces cerevisiae. Saccharomyces cerevisiae is the cheapest strain available for the conversion of biomass substrate. As conclusion, it was observed that with an increase in yeast quantity the ethanol yield increases reaching optimum yeast quantity then the ethanol yield start to decrease and the optimum ratio for molasses and water was 1:2. It can be concluded that the yield of ethanol is greatly dependent on the quantity of fermentable sugars present in the biomass.

ABSTRAK

Penggunaan biofuel sebagai alternatif kepada bahan api fosil telah berkembang dalam beberapa dekad yang lalu.Dalam tahun-tahun kebelakangan ini, perhatian semakin meningkat kepada penukaran biojisim kepada etanol bahan api, yang dianggap sebagai alternatif bahan api cecair yang bersih kepada bahan api fosil. Pengeluaran etanol (bioetanol) daripada biojisim merupakan salah satu cara untuk mengurangkan kedua-dua penggunaan minyak mentah dan pencemaran alam sekitar. Satu analisis keadaan semasa dan perspektif kepada biomas kepada etanol disediakan dalam kajian ini. Kajian ini juga berurusan terutamanya dengan hasil etanol dari molas berkenaan kepada nisbah pencairan dan jumlah yis yang digunakan untuk penapaian dengan menjaga pemalar suhu dan tempoh penapaian. Molases tebu akan digunakan untuk bahan mentah dalam kajian ini. Molases tebu sisa produk daripada pemprosesan tebu menjadi gula. Oleh itu, molases tebu seperti sisa pertanian adalah bahan mentah yang menarik untuk pengeluaran bioetanol. Sisa pertanian kos yang efektif, yang boleh diperbaharui dan melimpah. Dalam kajian ini, yis yang akan digunakan ialah Saccharomyces cerevisiae. Saccharomyces cerevisiae yang termurah untuk penukaran substrat biojisim. Selepas kajian ini, diperhatikan bahawa dengan peningkatan dalam kuantiti yis bertambah hasil etanol sehingga kuantiti yis optimum dicapai dan kemudian hasil etanol mula berkurangan dan nisbah optimum untuk molasses dan air adalah 1:2. Dapat disimpulkan bahawa hasil etanol adalah amat bergantung kepada kuantiti gula fermentasi hadir dalam biojisim.

TABLES OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

ATP	Adenosine Triphosphate
C_2H_5OH	Ethanol
CO_2	Carbon Dioxide
GPDH	Glucose-6-Phosphate Dehydrogenase
TRS	Total Reducing Sugar

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

One of the greatest challenges for society in the 21st century is to meet the growing demand for energy for transportation, heating and industrial processes, and to provide raw material for the industry in a sustainable way. In addition, the environmental deterioration resulting from the over-consumption of petroleum derived products, especially the transportation fuels, is threatening the sustainability of human society (Bai et al., 2008). Excessive consumption of fossil fuels, particularly in large urban areas, has resulted in generation of high levels of pollution during the last few decades. In this scenario, renewable sources such as wind, water, sun, biomass, geothermal heat can be the renewable sources for the energy industry whereas fuel production and the chemical industry may depend on biomass as an alternative source in the near future (Oliveira & Hira, 2009). All petroleum-based fuels can be replaced by renewable biomass fuels such as bioethanol, bio-diesel, bio-hydrogen, etc., derived from sugarcane, corn, switchgrass, algae, etc (Sarkar et al., 2011).

Bioethanol is regarded as one of the most promising biofuels from renewable sources. It is used for medicines, cosmetics, and industrial materials, and its production is increasing every year (Cardona & Sanchez, 2007). With increasing oil prices and global environmental concerns, bioethanol production has recently become a focus of attention (Bai et al., 2008). Ethanol is a volatile, flammable and colorless liquid that has a slight odor. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste. Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process.

Generally, bioethanol feedstock can be conveniently classified into three types which are sugar-based feedstock, starchy materials and lignocelluloses biomass (Razmovski, 2010), that show in Table 1.1. For this study, sugar-based feedstock, sugar cane molasses will be used as the feedstock. In particular, sugar-based feedstock contains readily available fermentable sugars and can be an ideal substrate for ethanol production by direct fermentation. Direct fermentation of sugars has advantages in production costs of ethanol, compared to processes that use starchy materials or lignocelluloses biomass as raw materials (Razmovski, 2010).

Table 1.1: Type of feedstock

Type of feedstock	Example
Sugar-based feedstock	Sugarcane, sugar beet, molasses, cane juice, beet juice
Starchy material	Wheat, corn, and barley
Lignocellulosic	Wood, straw, and bagasse

Molasses is a viscous by-product of of sugar cane process, grapes or sugar beets into sugar. First molasses was what was left after the sugar had been crystallized out once. When this was re-boiled and more sugar crystallized out, the remaining syrup was second molasses. After a third time, the molasses was blackstrap molasses. Blackstrap is still the result of the final boiling, which is why it’s less sweet and more strongly flavored and has a higher concentration of nutrients (such as iron and calcium) than other molasses. Molasses contains around 40% of sugar content that is fermented by yeast during the ethanol conversion process (Olbric, 2006).

1.2 Objectives

To produce ethanol from sugarcane molasses using yeast cells (Saccharomyces cerevisiae).

1.3 Scope of Study

In order to achieve the objective, the following scopes have been identified and to be applied:

(i) To study the amount of ethanol yield from sugarcane molasses fermentation process.

(ii) To investigate the effect of yeast quantity, temperature, and fermentation period on the ethanol yield using batch fermentation.

1.4 Problem Statement

Unlike fossil fuels, bioethanol is a renewable energy source produced through fermentation of sugars. Ethanol has already been introduced on a large scale in Brazil, the US and some European countries. It is expected to be one of the dominating renewable biofuels in the transport sector within the coming 20 years. A dramatic increase demand for bioethanol production was lead to this study. The major problem with bioethanol production is the availability of raw materials for the production and also the production cost. Therefore, sugar-based feedstock will be used as the feedstock. The other problem is to see whether sugarcane molasses can be used for the feedstock to produce the ethanol.

CHAPTER 2

LITERATURE REVIEW

2.1 Ethanol

Ethanol, also called ethyl alcohol, pure alcohol, grain alcohol, or drinking alcohol, is a volatile, flammable, colorless liquid. Ethanol, CH_3CH_2OH, is an alcohol, a group of chemical compounds which molecules contain a hydroxyl group, (OH^-), bonded to a carbon atom as shown in Fig. 2.1.

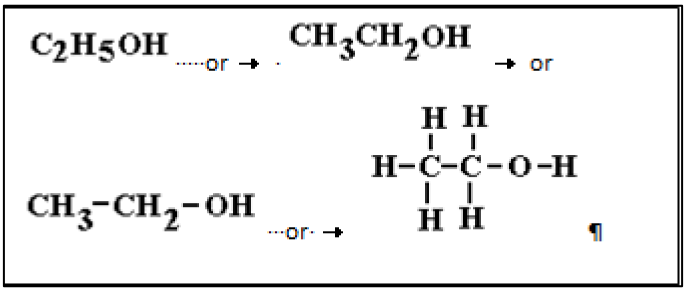

Fig 2.1: Ethanol structure

The word alcohol derives from Arabic al-kuhul, which denotes a fine powder of antimony used as an eye makeup. Alcohol originally referred to any fine powder, but medieval chemists later applied the term to the refined products of distillation, and this led to the current usage. Mostly, all the ethanol used industrially is a mixture of 95% ethanol and 5% water, which is known simply as 95% alcohol. Although pure ethyl alcohol is available, it is much more expensive and is used only when definitely required. Table 2 show the physical properties of ethanol (Ethanol, 2011).

Table 2.1: Physical properties of Ethanol

Properties	
Molecular formula	C_2H_6O
Molar mass	46.07 g mol−1
Exact mass	46.041864814 g mol−1
Appearance	Colorless liquid
Density	0.789 g/cm3
Melting point	−114 °C, 159 K, -173 °F
Boiling point	78 °C, 351 K, 172 °F

2.2 Feedstock for bioethanol production

Biofuels usually originated from plant oils, sugar beets, cereals, organic waste and the processing of biomass. Biological feedstock that contain appreciable amounts of sugar or materials that can be converted into sugar, such as starch or cellulose can be fermented to produce bioethanol to be used in gasoline engines (Kumar et al., 2006). Bioethanol feedstock can be conveniently classified into three types:

(i) sugar-containing feedstock (e.g. sugar beet, sweet sorghum and sugar cane),
(ii) starchy materials (e.g. wheat, corn, and barley), and
(iii) lignocelluloses biomass (e.g. wood, straw, and grasses) (Kumar et al., 2006).

Different feedstock that can be utilized for bioethanol production and their comparative production potential are given in Table 3 (Kumar et al., 2006).

Table 2.2: Different feedstock for bioethanol production and their comparative production potential.

Feedstock	Bioethanol production potential (L/ton)
Sugar cane	70
Sugar beet	110
Sweet potato	125
Potato	110
Cassava	180
Maize	360
Rice	430
Barley	250
Wheat	340
Sweet sorghum	60
Bagasse and other cellulose biomass	280

The availability of raw materials for the production is the one major problem for bioethanol production. The availability of feedstock for bioethanol can be considerably from season to season and also depend on geographic locations. Beside that, The price of the raw materials is also highly volatile, that can affect the production costs of bioethanol. Maximize the ethanol production is important because feedstock typically account for greater than one-third of the production costs (Balat et al., 2007).

2.2.1 Sucrose-containing feedstocks

Feedstock for bioethanol is essentially comprised of sugar cane and sugar beet. Two-third of world sugar production is from sugar cane and one-third is from sugar beet (Kumar et al., 2006). These two type of sugar are produced in geographically distinct regions. Sugar cane is grown in tropical and subtropical countries, while sugar beet is only grown in temperate-climate countries. World cane sugar export has not increased over the period 2000–2004 (Table 4) (Balat et al, 2007).

Table 2.3: Evolution of world exports of raw cane sugar

	2000	**2001**	**2002**	**2003**	**2004**
Value ($ billion)	3.2	4.3	2.8	3.4	2.9
Quantity (million tons)	16.5	17.9	12.9	16.7	14.5

Brazil is the largest single producer of sugar cane with about 27% of global production and a yield of 18 metric tons of dry sugar cane per hectare highest yield occurs in Peru, which produces more than 32 metric tone of dry sugar cane per hectare (Kim & dale, 2004). Bioethanol production from sugar cane is very economical in Brazil because of two primary reasons. Brazil dropped support of sugar prices to support the bioethanol industry with government established mandates for the blending of bioethanol with gasoline. This drastically lowered the cost of the feedstock, sugar cane, and created a demand for and supported the price of bioethanol (Shapouri et al., 2006). The Brazilian bioethanol industry was poised for a major jump during 2006–2008 as a part of new national plan to increase sugar cane production by 40% by 2009 (Balat et al, 2007).

In European countries, beet molasses are the most utilized sucrose-containing feedstock (Cardona & Sanchez, 2007). Sugar beet crops are grown in most of the EU-25 countries, and yield substantially more bioethanol per hectare than wheat (Balat et al., 2007).The advantages with sugar beet are a lower cycle of crop production, higher yield, and high tolerance of a wide range of climatic variations, low water and fertilizer requirement. Compared to sugar cane, sugar beet requires 35–40% less water and fertilizer (Kumar et al., 2006).

2.2.2 Starchy materials

Another type of feedstock that can be used for bioethanol production is starch-based materials (Sorapipatana, 2007). Starch is a biopolymer and can be defined as a homopolymer consisting only one monomer, d-glucose (Pongsawatmanit et al., 2007). The chains of this carbohydrate must be break down to obtaining glucose syrup, which can be converted into bioethanol by yeasts. This type of feedstock is the most used for bioethanol production in North America and Europe. Corn and wheat are mainly employed with these purposes (Cardona Sanchez, 2007).

The United States has a large corn-based bioethanol industry with a capacity of over 15 billion per year, production capacity is anticipated to continue rising to about 28 billion per year by 2012, as dictated by the Energy Policy Act of 2005 (Mabee et al., 2006). The bioethanol industry used more than 1.4 billion bushels (1 bushel = 56 pounds) of corn in 2005, valued at $2.9 billion (Balat et al., 2007). The availability of feedstock is not expected to be a constraint for bioethanol production over the next decade. Corn, which is currently used to make about 90% of all US bioethanol, is expected to remain the predominant feedstock, although its share likely will decline modestly by 2015. As corn stocks are drawn down from this season 2.4 billion bushel projected carryout, farm-level corn prices will increase, reaching $2.58 per bushel by the 2015 marketing year. The impact of this level of demand for bioethanol on stocks measured by the stocks to use ratio and farm-level corn prices is illustrated in Fig. 2.2 (Urbanchuk, 2006).

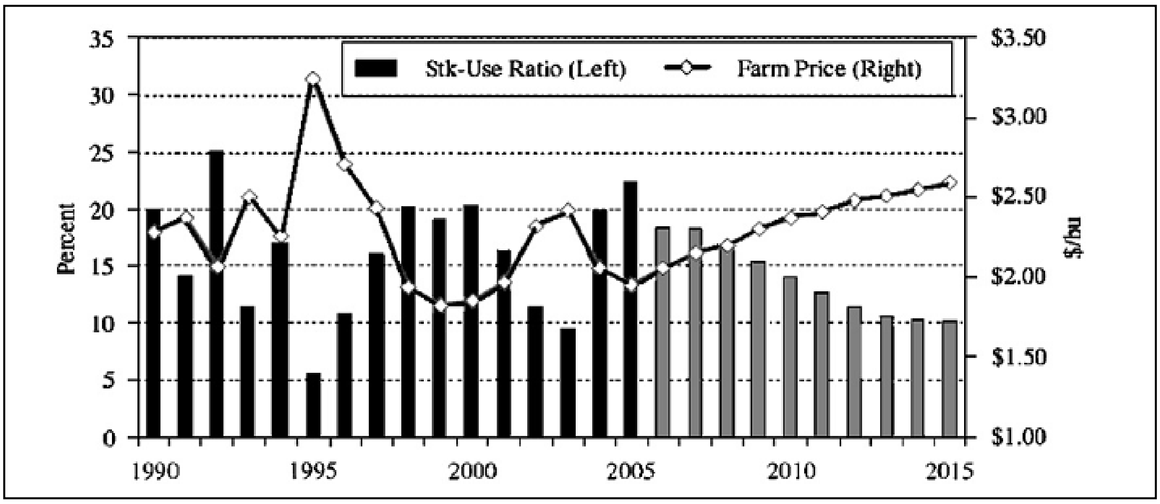

Figure 2.2: US corn stocks and farm price

The greatest cost in the production of bioethanol from corn, and the cost with the greatest variability, is the cost of the corn. Corn prices vary from year to year and in the last few years have ranged from \$1.94 / bushel to \$3.24 / bushel. The price of corn in the US is now close to \$4.00 / bushel. Corn prices will also vary in different locations due to shipping distance from the field to the plant (McAloon et al., 2000).

Considering that corn transportation to distilleries requires 0.63 gigajoule (GJ) per m^3 of bioethanol produced, and that bioethanol conversion consumes 13.7 GJ of energy per m^3 of bioethanol produced in situ, the resulting energy output–input ratio for US bioethanol production is 1:1, which is significantly lower than the ratio of 3:7 for Brazilian bioethanol from sugar cane. Transportation, refinery and cleanout costs were excluded for on-site systems (Oliveria et al., 2005).

Starch consists of long chains of glucose molecules and can also be converted to fermentable sugar by a method called “the hydrolysis technique”. Hydrolysis is a reaction of starch with water, which is normally used to break down the starch into fermentable sugar (Sorapipatana & Yoosin, 2007). There are two types of hydrolysis which it enzymatic hydrolysis and acid hydrolysis. The hydrolysis of starch by amylases at relatively high temperatures is a process known industrially as liquefaction. The factors that affect the enzymatic hydrolysis of starch include substrates, enzyme activity, and reaction conditions (temperature, pH, as well as other parameters) (Neves, 2006). The starch-based bioethanol industry has been commercially viable for about 30 years; in that time, tremendous improvements have been made in enzyme efficiency, reducing process costs and time, and increasing bioethanol yields (Mabee et al., 2006). There are two main reasons for the present high cost: one is that, as the yeast Saccharomyces cerevisiae cannot utilize starchy materials, large amounts of amylolytic enzymes, namely, (glucoamylase and α-amylase), need to be added; the other is that the starchy materials need to be cooked at a high temperature (413–453 ^{o}K) to obtain a high bioethanol yield(Shigechi H, 2004).

2.2.3 Lignocellulosic biomass

Lignocelluloses biomass, such as agricultural residues (corn stover and wheat straw), wood and crops, is an attractive material for bioethanol fuel production since it is the most abundant reproducible resource on the Earth. Lignocelluloses biomass could produce up to 442 billion per year of bioethanol (Bohlmann, 2006). Thus, the total potential bioethanol production from crop residues and wasted crops is 491 billion per year, about 16 times higher than the current world bioethanol production (Kim & Dale, 2004). In addition, rice straw is one of the abundant lignocelluloses waste materials in the world. It is annually produced about 731 million tons, which is distributed in Africa (20.9 million tons), Asia (667.6 million tons), Europe (3.9 million tons), America (37.2 million tons) and Oceania (1.7 million tons) (Balat et al., 2007).

Lignocelluloses perennial crops (e.g. short rotation coppices and inedible grasses) are promising feedstock because of high yields, low costs, good suitability for low quality land (which is more easily available for energy crops), and low environmental impacts (Balat et al., 2007).

2.2.4 Ethanol from cane molasses

Molasses is generally used because it is rich in all salts except nitrogen which is normally employed in the actual growth of yeast cells. Molasses is defined as waste product of sugar industry of which further extraction of sugar is uneconomical, contains about 45–50% fermentable sugars (Olbric, 2006). Because of the ease with which this can be fermented into ethanol and its low price have made this raw material ideal for ethanol production. In recent years and because of decontrol, a jump in molasses price and limitation on molasses availability, ethanol production has been greatly affected in molasses-based distilleries (Nguyen & Gheewala, 2008).

Molasses are the final effluent (final molasses) and by-products of sugar manufacture. Due to their origin, sugar cane and molasses are different. Black strap molasses are molasses produced from raw sugar factories from cane or beet. Often, only term (molasses) is used for molasses from a beet factory where as black strap is preferably used for cane molasses. Refinery (final) molasses (refinery black strap, barrel syrup), is derived mainly from cane refineries, where white sugar is produced from raw sugar. The Figure 1 shows the process of sugar manufacturing.

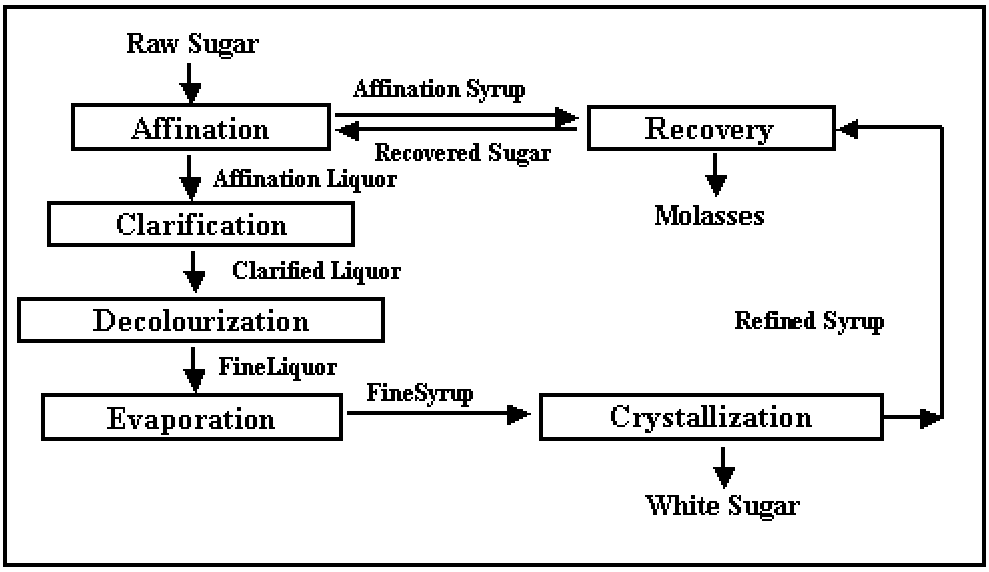

Figure 2.3: Sugar refinery process

Molasses is a dark reddish colored jelly like viscous material. The pH of molasses varies from 6.8 to 8.5. The high osmotic pressure of molasses protects it from microbial spoilage, and it can be easily transported by barge and tankers. Molasses can be pumped easily. For production of ethanol in non-sugar producing areas, and particularly in areas with suitable waterways for barge shipment, molasses may be the best suitable raw material for fermentation. Molasses are classified into following three grades :

a) First grade Molasses: It contains more than 50% total reducing sugar (TRS).
b) Second grade Molasses: It contains 40%to 50% total reducing sugar (TRS).
c) Third grade Molasses: It contains less than 40% total reducing sugar (TRS).

General composition of cane molasses: Molasses is an agricultural product and its composition varies with the variety of maturity of cane with the climate and soil condition. In addition processing condition in the sugar factory may also bring about changes in the composition of molasses. So, only average values of main components of cane black strap molasses show in table 2.4.

Table 2.4: Main components of cane black strap molasses

Composition	Percentage
Sugars	73.1
Sucrose	45.5
Invert sugar	22.1
Other	5.5
Organic	15.5
Glutamic acid and pyrollidine carboxylic acid	2.4
Other N	3.1
Organic acids	7.0
Pectin etc.	2.7
Inorganic	11.7
K_2O	5.3
Na_2O	0.1
CaO	0.2
MgO	1.0
SO_2+SO_3	2.3
P_2O_5	0.8
Others	0.9

2.3 Processes of ethanol production

The raw materials that contain sugars, or materials which can be transformed into sugars, can be used as fermentation substrates. The fermentable raw materials can be grouped as directly fermentable sugar materials, starchy, lignocellulose materials and urban or industrial wastes. Sugar containing materials require the least costly pretreatment, where starchy, lignocellulose materials and urban/industrial wastes needed costly pretreatment, to convert into fermentable substrates (Sun and Cheng, 2002). Sugar containing materials which can be transformed into glucose, can be used as fermentation substrates under anaerobic conditions, glucose is converted to ethanol and carbon dioxide by glycolysis. The phosphorylation of carbohydrates is carried out through the metabolic pathway and the end products are two moles of ethanol and carbon dioxide (Ingram et al, 1998).

Although fungi, bacteria, and yeast microorganisms can be used for fermentation, specific yeast (S. cerevisiae also known as Bakers' yeast, since it is commonly used in the baking industry) is frequently used to ferment glucose to ethanol. Theoretically, 100 g of glucose will produce 51.4 g of ethanol and 48.8 g of carbon dioxide. However, in practice, the microorganisms use some of the glucose for growth and the actual yield is less than 100% (Lin & Tanaka, 2005).

Ethanol production from grain involves milling of grain, hydrolysis of starch to release fermentable sugar, followed by inoculation with yeast. Chemically starch is a polymer of glucose (Marina et al., 2009). Yeast cannot use starch directly for ethanol production. Therefore, grain starch has to be wholly broken down to glucose by combination of two enzymes, viz., amylase and amyloglucosidase, before it is fermented by yeast to produce ethanol. The biochemical reactions and processes involved in starch hydrolysis and fermentation which is given in Eq. 1 + Eq. 2 and shown in Fig. 2.3 and Fig.2.4. Alcohol produced from fermented broth and remaining spillages is processed to produce Distiller's Dried Grain and Soluble (DDGS), which is an excellent ingredient for animal feed (Marina et al., 2009).

$(C_6H_{10}O_5)n + nH_2O \rightarrow nC_6H_{12}O_6$ .... Eq.(1)

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ + energy (stored as ATP) .... Eq.(2)

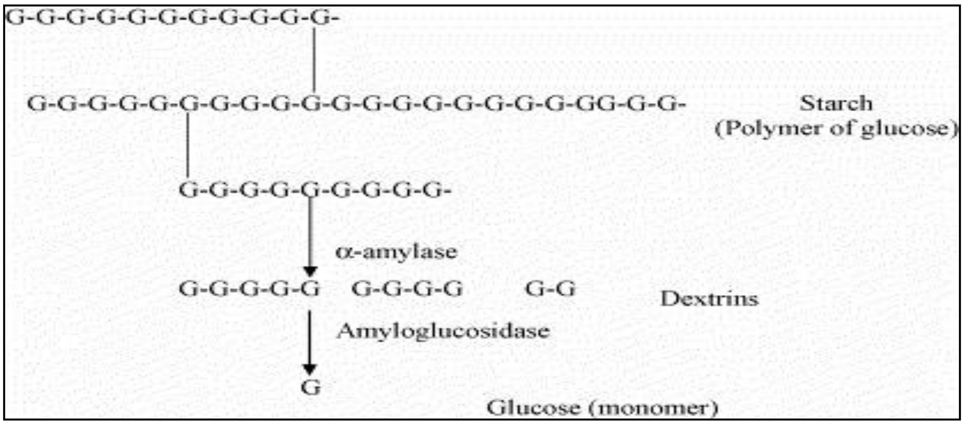

Fig. 2.4: Enzymatic hydrolysis of starch to glucose.

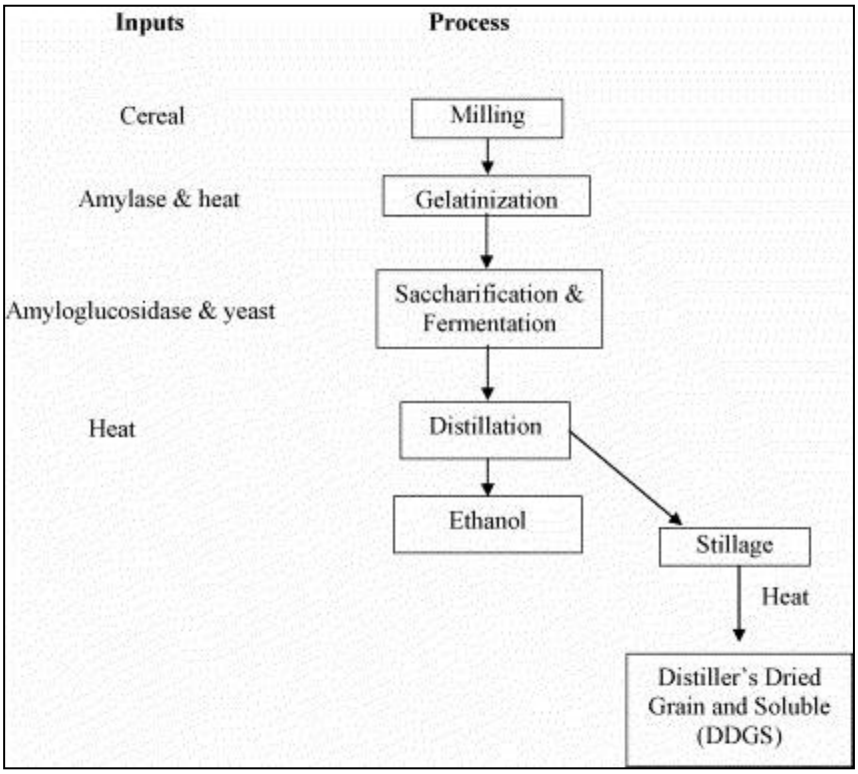

Fig. 2.5: Flow chart of ethanol production from cereal grains.